Workshop H
Macrophages, Neutrophils and Cytokines

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H.1 Interleukin-4 enhances the interferon-γ-mediated activation of murine macrophages for killing of *Leishmania major* amastigotes

Christian Bogdan, Steffen Stenger, Martin Röllinghoff, and Werner Solbach

In murine cutaneous *Leishmania major (L. major)* infections susceptible mice (e.g. BALB/c) have been found to produce high levels of interleukin-4 (IL-4), but only small amounts of interferon-γ (IFN-γ) in the course of the disease. In resistant mice (e.g. C57BL/6) a reciprocal expression of the cytokines has been described. We therefore investigated the effect of recombinant murine IL-4 in the absence or presence of recombinant murine IFN-γ on adherent bone-marrow macrophages (MΦ), peritoneal-exudate or resident peritoneal MΦ, which were pulse-infected with *L. major* amastigotes. IL-4 (5–100 U/ml) failed to activate any of these MΦ populations for killing of intracellular amastigotes, no matter whether the cytokine stimulation was started prior to or only after infection of the MΦ-cultures with *L. major* parasites. However, in the presence of low doses of IFN-γ (10–20 U/ml), which alone only caused a slight reduction of the number of intracellular parasites, IL-4 led to a highly significant acceleration of the parasite elimination by bone marrow or peritoneal exudate MΦ as compared to the control cultures. In the case of resident peritoneal MΦ the synergistic effect of IFN-γ and IL-4 was observed only when the MΦ were stimulated with both cytokines or IFN-γ alone for at least 10 hours prior to infection; adding both cytokines after infection of the MΦ did not lead to a significant reduction of the intracellular parasite burden. The synergistic effect of IL-4 and IFN-γ was completely abrogated in the the presence of anti-IL-4 antibodies. Furthermore, there was no significant difference between MΦ-cultures derived from susceptible and those from resistant mice. From these data we conclude that IL-4 is not an IFN-γ-antagonizing cytokine, but provides a strong stimulus for the killing of intracellular *L. major* amastigotes in the presence of suboptimal dosages of IFN-γ. Also, IFN-γ appears to provide an important priming signal for the activation of resident MΦ to eliminate intracellular amastigotes. On the basis of our experiments susceptibility to a disseminating *L. major* infection is linked to the lack of IFN-γ rather than to the excessive amount of IL-4.

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H.2 Effects of sodium fluoride on mediator release from human neutrophils and platelets

Christine Brom and Wolfgang König

The release of inflammatory mediators by human neutrophils (PMN) or platelets in response to receptor-mediated stimulation and/or unspecific G-protein activation by sodium fluoride (NaF) was analyzed. The incubation with NaF resulted in a dose- and time-dependent generation of leukotrienes (LT) or 12-HETE, respectively. Pretreatment of cells with pertussis toxin or cholera toxin had no influence on the NaF-induced mediator release. The metabolism of LTB_4 to 20-COOH-LTB_4 is inhibited by NaF. Combined stimulation of PMNs (1×10^7 cells) with NaF (20 mM) and FMLP (10^{-5} M) showed a synergistically enhanced LT-generation. Platelets (1×10^8 cells) preincubated with thrombin (2 U/ml) followed by stimulation with NaF showed a reduced release of 12-HETE. No correlations between mediator release and chemiluminescence by PMNs or platelet aggregation were observed. However, binding of Gpp(NH)p to G-proteins of membrane fractions of NaF- or FMLP-preincubated PMNs correlated with the respective LT-release. Gpp(NH)p-binding by platelet membranes showed no correlation to their ability for aggregation or release of 12-HETE. We suggest that, via similar mechanisms of stimulation, within different cell populations either diverse control mechanisms or distinct sets of G-proteins exists, leading to inhibitory or stimulatory cell responses.

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H.3 Interleukins and signal transduction in human neutrophils

Joachim Brom, Christine Brom, and Wolfgang König

Preincubation of human neutrophils (PMN) with IL-3 or IL-8 resulted in an increased FMLP-induced chemiluminescence response. In order to study the biochemical basis of the "priming" phenomenon, the interactions between various interleukins and the signal transduction cascade or initial cellular events were analyzed. Incubation of PMN (5×10^6 cells) with IL-3, IL-8 or TNF-β for 15 sec resulted in the polymerization of cytoplasmic G-actin to F-actin as was determined following FITC-phalloidin staining. Combined stimulation with the receptor-mediated stimulus FMLP (10^{-8} M) synergistically increased the content of F-actin. Furthermore, GTPase activity as a parameter of G-protein involvement was measured with membrane fractions. IL-3, IL-8 and TNF-β alone showed no effects on GTPase activity, but in combination with FMLP a significant enhancement of GTP-hydrolysis was observed. Determination of specific FMLP-binding sites after interleukin preincubation indicated that the increased cellular responses were not the result of an enhanced receptor expression. The data presented suggest that the «priming» of neutrophils is most likely the result of the preactivation of diverse signal transduction elements, e.g. the cytoskeletal organization or the involvement of G-proteins.

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H.4 C1q, the first component of complement binds only to activated human platelets

D. Elendt, Kathrin Kirsch, and K. Malberg

Human platelets possess specific binding sites for human C1q, the first component of complement. We tested whether this receptor is dependent on platelet activation like the well known fibrinogen receptor.

Non activated platelets were prepared by gel filtration over Sepharose CL-2B equilibrated with a medium that contained dipyridamol, EDTA and hirudin and fixed with formaldehyde. Activated platelets were prepared by centrifugation, thrombin activation and fixation with formaldehyde.

Activation or non activation was controlled by SEM. C1q binding to platelets was first shown by a three-step-procedure on slides. Platelets were successively incubated with biotinylated C1q, avidin and biotinylated horse radish peroxidase. The binding was then quantified with radioiodinated C1q. Activated platelets bound 6000 ± 3600 molecules per platelet with an apparent dissociation constant of $4.4 \times 10^{-8} \pm 2.4 \times 10^{-8}$ molar. Non activated platelets bound very low amounts of C1q so that binding could not be quantified.

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H.5 Macrophages regulate intracellular glutathione levels of lymphocytes. Evidence for an immunoregulatory role of cysteine

Helmut Gmünder, Hans-Peter Eck, Bernd Benninghoff, Steffen Roth, and Wulf Dröge

Macrophages consume cystine and generate approximately equivalent amounts of acid soluble thiol. Stimulation of macrophages with bacterial lipopolysaccharide (LPS) or tumor necrosis factor (TNF) strongly augments the amount of thiol released into the culture supernatant. Cysteine constitutes most of the acid soluble thiol. The intracellular glutathione level and the DNA synthesis activity in mitogenically stimulated lymphocytes are strongly increased either by exogenously added cysteine, or by (syngeneic) macrophages. This cysteine dependency is observed even in the presence of relatively high extracellular cystine concentrations as they occur in the blood plasma. The extracellular cysteine concentration has also a strong influence on the intracellular glutathione concentration, viability, and DNA synthesis of cycling T cell clones and on the late phase (day 3 and 4) of a 5 day allogeneic mixed lymphocyte culture with respect to the generation of cytotoxic T cell activity, indicating that cysteine regulates not only the early phase of the blastogenic response. The inhibitory effect of cysteine starvation on the DNA synthesis of the T cell clones and on the activation of cytotoxic T lymphocytes can be explained essentially by the depletion of intracellular glutathione, since similar effects are observed after treatment with buthionine sulfoximine (BSO), a specific inhibitor of the glutathione biosynthesis. BSO has practically no influence, however, on the BLT-esterase activity and hemolytic activity of the cell lysates from cytotoxic

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T cells against sheep red blood cells (perforin activity). Taken together, our experiments indicate that cysteine has a regulatory role in the immune system analogous to the hormone-like lymphokines and cytokines. It is released by macrophages at a variable and regulated rate and regulates immunologically relevant functions of lymphocytes in the vicinity.

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H.6 A membrane-bound form of the acute phase protein CRP is the galactose-specific particle receptor on rat liver macrophages

VICTORIA KOLB-BACHOFEN and GRAZYNA KEMPKA

Rat liver macrophages express a galactose-specific receptor which mediates endocytosis of particles or neuraminidase-treated blood cells. From rat serum we now have isolated a galactose-specific lectin by affinity chromatography. Comparative analysis of this serum galactose-binding protein (SGL) with the galactose-particle receptor protein purified from rat liver macrophages and with the acute phase protein C-reactive protein (CRP) reveals close relation or identity of these proteins. An apparent molecular weight of 30 kD was determined for all three proteins by SDS-PAGE under reducing conditions and of about 130 kD by native PAGE. All three proteins exhibit the same pentameric, ring shaped structure. Antibodies raised against the serum galactose-binding protein or against the macrophage receptor cross-react. Monoclonal antibodies raised against rat CRP label liver macrophage but not hepatocyte surfaces and react with all three isolated proteins in a Western blot assay. Furthermore, the galactose-particle receptor can be functionally replaced by purified C-reactive protein. Northern blot analysis showed that the CRP is not synthesized in the macrophages but appears to be acquired from hepatocytes or blood. We now conclude, that a membrane-bound form of CRP functions as the recycling galactose-particle receptor in rat liver Kupffer cells.

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H.7 Biochemical properties of endogenously synthesized MHC-class II-molecules derived from rat Langerhans cells

U. NEISS[1], D. BECKER[1], J. KNOP[2], and K. RESKE[1]

Epidermal Langerhans cells (LC) play a fundamental role in the initiation of an immune response in the skin. In contrast to human and mouse LC less data are available on rat LC. In an attempt to define the cell surface phenotype of rat LC more precisely we assessed various cell surface markers by immunocytological staining and FACS analysis. Expression of the RT1.B- (I-A) and RT1.D- (I-E) isotypes, the leukocyte-common-antigen (L-CA), the C3bi-receptor, including additional markers that are found on macrophages has been observed. To elucidate the relationship of polymorphic and invariant chains, endogenous synthesis of MHC-class II-molecules was measured by metabolic labeling of enriched and non-enriched LC. Immunoprecipitation analysis of LC-derived MHC-class II-molecules using mAb OX3

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and OX6, followed by 2-dimensional gel electrophoresis revealed spot patterns which resembled corresponding patterns found in other constitutively Ia-positive cells. mAb OX3 selectively precipitates the terminally glycosylated α,β heterodimer, whereas mAb OX6 precipitates γ chain deficient and γ chain containing polypeptide chain complexes.

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H.8 Characterization of genomic FcγRIII (CD16) clones

Patricia Pring-Åkerblom, Waldemar Kolanus, and R. E. Schmidt

The CD16 antigen is expressed on human NK cells, macrophages and PMN. It has been shown to mediate ADCC by NK cells, where it represents the only FcγR (1). On PMN, CD16 has been proposed to act together with CD32 to mediate effector functions. Recently it has been shown that FCγRIII is anchored through PI-linkage on PMN, whereas the NK isoform is a transmembrane protein. The CD16 polymorphism was shown to be based on mutually exclusive expression of two separate genes in NK cells and granulocytes (2). Therefore it can be hypothesized that differential expression of CD16 mRNA is due to tissue-specific regulation of transcription. In order to characterize putative control regions of the CD16 genes, a genomic library of human placenta DNA was constructed in EMBL3. Using a ^{32}P-labeled CD16 cDNA probe, thirteen genomic clones were obtained by plaque hybridization and subsequent purification. Restriction mapping revealed that three of these clones included several kb of the 5′-upstream region. According to known restriction site polymorphisms and preliminary sequence analysis, two of these clones could be assigned to the PMN CD16 allele Na-2, whereas the third one represents the NK gene. Complete sequencing of the 5′ regions and functional analysis of putative control elements will help to elucidate the mechanisms of tissue-specific expression of CD16.

1. Werfel, T., Uciechowski, P., Tetteroo, P. A. T., Kurrle, R., Deicher, H., and Schmidt, R. E. (1989): Activation of Cloned Human Natural Killer Cells via Fcγ RIII. J. Immunol 142: 1102–1106.
2. Ravetch, J. V., and Perussia, B. (1989): Alternative Membrane Forms of Fcγ RIII (CD16) on Human Natural Killer Cells and Neutrophils. J. Exp. Med. 170: 481–497.

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H.9 Regulation of the constitutive IL-6 expression of human glomerular mesangial cells (HMC)

H. H. Radeke, J. von der Ohe, and K. Resch

Recent studies demonstrated that human mesangial proliferative glomerulonephritis is characterized by urinary IL-6 excretion and mesangial IL-6 expression.

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We studied IL-6 secretion of cultured HMC measuring the IL-6 dependent proliferation of the mouse B-cell hybridoma, 7TD1 (van Snick) after 72 h incubation by MTT staining. The specificity of this cell line for mouse and human IL-6 had been well established. Unstimulated MHC constitutively secreted IL-6 bioactivity and dilutions down to 1:512 caused significant 7TD1 proliferation. This corresponds to an IL-6 activity of 100 U/ml equivalent to 1 ng/ml rhIL-6. To analyse the effect of different stimuli in series of experiments we calculated a stimulation index (S.I., i.e. the 7TD1 growth stimulation induced by a 1:16 dilution of HMC supernatants divided by the respective assay control). IL-1-β (25 ng/ml) stimulation of HMC for 2, 4, 8, 12, 24, 48 hrs significantly increased IL-6 secretion after 8 hrs (5.09 ± 0.79 S.I. vs. 1.87 ± 0.05 S.I. of unstimulated HMC). The peak response towards IL-1-β and TNF-α (10 ng/ml) was measured after 24 hrs being 6.58 ± 0.91 S.I. and 2.56 ± 0.24 S.I., respectively. Combinations of IL-1-β and TNF-α resulted in a marked synergistic induction of IL-6 especially at lower concentrations. IL-1-β (12.5 ng/ml) gave a S.I. of 3.32 ± 0.57, TNF-α (5 ng/ml) 1.33 ± 0.05, whereas the combination resulted in 9.19 ± 1.01 S.I. Other potent activators of IL-6 secretion by HMC were IL-1-α, LPS and stimulated monocyte supernatants, whereas IL-2, IFN-gamma and supernatants of stimulated Jurkat cells had no effect on IL-6 secretion. In conclusion, the high basal IL-6 production of HMC possibly correlated to the proliferative status could be further enhanced by monocytes factors. This may be important for the progression of human mesangio-proliferative GN.

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H.10 Immunohistological demonstration of two calcium binding proteins MRP-8 and MRP-14 in experimentally induced dermatitis

J. Roth, C. Sunderkötter, S. Teigelkampf, and C. Sorg

MRP-8 and MRP-14 are two calcium-binding proteins belonging to the S-100 family. They are expressed during differentiation of granulocytes and monocytes/macrophages. We performed immunohistological studies on the distribution of MRP-8 and MRP-14 positive cells during experimentally induced dermatitis in mice. During allergic contact dermatitis in BALB/c mice (DNFB-challenge on ears of sensitized animals) we observed a marked increase of MRP-8 and MRP-14 positive cells in the resulting infiltrate. Their percentage reached a maximum after 1 to 3 days (MRP-8: 60% and MRP-14: 80% of total cell number) and declined to normal values (< 5%) after 2 weeks. During irritant contact dermatitis (challenge with 1% croton oil) maximal infiltration with MRP-8 and MRP-14 positive cells was already present after 8 hrs to 1 day and declined drastically after 2 days reaching normal values after one week. Allergic and irritant contact dermatitis were also elicited in C57BL/6 mice which are known to be low responders in different models of inflammation. In this strain the increase of MRP-8 and MRP-14 positive cells in both types of contact dermatitis was similar to that seen in BALB/c mice. The subsequent decrease, however, was observed significantly earlier. In both strains the expression of MRP-8 and MRP-14 correlated with the advent of macrophages and the intensity of the infiltrate.

Conclusions: 1. Expression of MRP-8 and MRP-14 correlates with the activity of inflammatory processes. 2. Our results indicate that the high sensitivity of BALB/c mice to inflammatory stimuli is caused by an altered differentiation of monocytes/macrophages.

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H.11 Evaluation of the hetrazepine WEB 2086BS, a specific PAF-antagonist, to the xenogeneic response during hyperacute renal rejection

D. M. Saumweber, R. Bergmann, J. Gokel, and C. Hammer

Platelet-activating-factor (PAF) is a potent phospholipid mediator elaborated by a variety of mammalian cells. PAF has been shown to be released during immune responses, e.g. graft rejection. The aim of this study was to investigate whether the specific PAF-antagonist WEB 2086BS is able to influence the hyperacute rejection of kidneys (HXAR) in an *ex vivo* xenograft model.

Pig kidneys were perfused *ex vivo* with freshly taken autologous porcine blood (group 1: n = 8), xenogeneic human blood (group 2: n = 8) or xenogeneic human blood treated with WEB 2086BS (group 3: n = 8) respectively. Fractions of the perfusate were collected up to 60 min after start of hemoperfusion. Hemogram, perfusion pressure, absorption of preformed natural antibodies, urine and plasma osmolality were measured. To determine the release of immunoreactive and vasoactive substances acting during HXAR, the anaphylatoxins C3a and C4a, the eicosanoids TXA2 and PG12, as well as the cytokine TNF were measured by RIA.

Though the absorption of PNAB in both xenogeneic perfused groups was comparable, lower levels of C4a, TNF and the arachidonic acid metabolites TXA2 and PG12 were detected in the WEB 2086 treated group 3 vs the non treated group 2. As an indicator of kidney function the ratio of urine/plasma osmolality showed loss of kidney function in group 2 in contrast to kidneys of group 3, which were able to concentrate in comparable fashion to kidneys perfused with autologous porcine blood (group 1) at the whole observation time. While leucocytes and platelets decreased dramatically (even more pronounced in group 2 vs group 1 and 3), the perfusion pressure was consistently maintained at significantly higher levels in group 2 compared to all others.

Out data indicate that in this *ex vivo* xenograft model, the activation of the complement cascade, and the mediator response to hyperacute xenogeneic rejection could be delayed by the PAF-antagonist WEB 2086BS. This confirms the central role of PAF in HXAR, suggesting that hyperacute xenogeneic rejection might be delayed by polydirectional treatment with various specific mediator antagonists to a clinically relevant longterm xenograft survival.

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H.12 Activation of human granulocyte functions by hemolysin producing bacteria and modulation by interleukin-6

J. Scheffer, B. König, and W. König

We provided evidence that mediators of inflammation, e.g. oxygen radicals, enzymes and leukotrienes, are released from human granulocytes upon interaction with different bacterial hemolysins. A variety of genetically cloned strains which differed in hemolysin expression and release were studied. Stimulation with hemolysin producing bacteria (*E. coli* K12 pANN5211,

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S. marcescens W1436, *S. aureus* 121C) induce an inflammatory response. Mediators of these reactions include oxygen radicals, granular enzymes and leukotrienes. Furthermore, we studied the modulation of chemiluminescence response, enzyme release, phagocytosis and leukotriene formation from human granulocytes mediated by exogenous interleukin-6 (IL-6). The target cells were incubated with human recombinant interleukin-6 and stimulated with the hemolysin producing bacteria. The results obtained showed a 20–35 % reduction of most granulocyte functions after stimulation with the hemolysin producing *E. coli* K12 pANN5211. A decreased phagocytosis and leukotriene formation was observed. Stimulation with *S. aureus* 121C reduced the chemiluminescence response and phagocytosis after preincubation with IL-6. An inhibition of leukotriene formation was not observed. In contrast to bacterial stimulation, IL-6 did not alter the granulocyte activation after stimulation with the calcium-ionophore A23187. Our data indicate that interleukin-6, a mediator which is produced several hours after infection, may suppress an over-exaggerated response of inflammatory reactions from human granulocytes after stimulation with hemolysin producing bacteria.

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H.13 Cell-free supernatants of mitogen treated thymocytes promote the differentiation of mouse bone marrow cells into dendritic cells

Ch. Schleicher, R. Zecher, and K. Reske

Dendritic cells (DC) represent a minor population of bone marrow derived leukocytes with distinct morphology and function. These cells constitutively exhibit high levels of MHC class II antigens. They are extremely potent accessory cells and are known to elicit strong allogeneic and syngeneic mixed leukocyte reactions. DC occur in numerous tissues in very low numbers (< 1 %). To examine the properties of these cells one has to rely on certain isolation procedures which are rather inefficient. We therefore attempted to work out an *in vitro* cell culture system to obtain DC without cell fractionation. By the use of cell-free supernatants of mitogen induced thymocytes we succeeded in establishing such an *in vitro* system which leads to the differentiation of elevated numbers of mouse bone marrow cells with dendritic function and morphology. Data on these cells and their functional behavior will be presented.

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H.14 Studies on the metabolism of leukotriene B_4 in differentiated human monocytes and U937 cells

W. Schönfeld, Sabine Kasimir, J. Knöller, and W. König

Leukotriene B_4 has important proinflammatory functions such as chemotaxis and modulation of cytokine production in lymphocytes and macrophages. We previously reported that human alveolar macrophages not only synthesize this lipidmediator but in addition metabolize leukotriene B_4 (LTB_4) rapidly into the dihydro-LTB_4 by reduction of one of the conjugated double bonds. However, studies on peripheral monocytes indicated that these cells convert

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LTB_4 to less than 5 % into dihydro-LTB_4 within 60 min. In contrast, monocytes cultured for 24 h – 5 days had an increased capacity to metabolize LTB_4. A similar pattern was observed in the human monocytic cell line U937. Undifferentiated cells did not metabolize LTB_4. Within 5 h after the addition of dimethyl sulfoxide metabolism of LTB_4 could be observed. This was completely blocked in the presence of cycloheximide, suggesting that the LTB_4 reductase was newly synthesized during the differentiation process. In contrast, the myeloid HL-60 cells expressed a highly active LTB_4-reductase in the undifferentiated state. Studies with cell homogenate indicated that the activity of the enzyme depended on the presence of NADPH, Ca^{2+}, Mg^{2+} with an pH optimum at 7.5. Purification by subcellular fractionation and solubilization revealed evidence that the enzyme was located within the cellular membrane.

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H.15 Analysis of the CD31 antigen, a molecule which is structurally and functionally associated with Fc receptors

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In an attempt to identify signal transducing surface structures of human granulomonocytic cells, we established two monoclonal antibodies, termed 1B5 and 7E4, which after binding to monocytes but not to granulocytes induced the generation of reactive oxygen metabolites. The biochemical and cellular analyses of the antigens recognized by the two monoclonal antibodies revealed that both antibodies are directed against the CD31 molecule. In particular, this was confirmed by cloning the cDNA encoding the CD31 antigen. Sequence comparisons and quantitative measurements of the relatedness of the CD31 antigen showed that the extracellular segment of the molecule consists of six immunoglobulin-like constant domains and that each domain is substantially related to one or more domains drawn from IgG-Fc receptors. This finding suggested that the CD31 antigen might have immunoglobulin Fc binding capacity. By using CD31 transfectants and immunoglobulin coated beads this assumption could not be affirmed. However, Fc receptors turned out to be involved in the mechanism for activation of the monocyte respiratory burst via CD31. Details of the biochemical and functional analyses will be presented.

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H.16 Early differences in the cellular infiltrate between resistant and susceptible mouse strains after infection with *Leishmania major*

C. Sunderkötter, W. Beil, H. Bildau, J. Gutwald, and C. Sorg

In murine leishmaniasis studies have focussed primarily on the specific T-cell response (proliferation of protective T_H1-cells in resistant mice and of non protective T_H2-cells in susceptible strains).

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We investigated the early cellular events which precede the stage of specific T-cell response. After subcutaneous injection of *Leishmania major* into footpads of resistant C75BL/6 and susceptible BALB/c mice we performed immunohistochemical studies on the early infiltrate. We found that after two days the number of specifically stained, mature macrophages is increasing much faster in resistant mice than in susceptible mice.

Bone marrow cultures of the two mouse strains revealed that the earlier appearance of macrophages in the lesion of C57BL/6 mice correlates well with a more rapid maturation of bone marrow macrophages in this resistant strain.

We conclude that one reason for a more efficient control of *Leishmania* infection by C57BL/6 (as compared to BALB/c) may lie in a more rapid, T-cell independent maturation of macrophages.

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H.17 Production of bFGF or TNF-α by macrophages does not appear mandatory for the induction of angiogenesis

C. Sunderkötter, J. Roth, and C. Sorg

TNF-α and bFGF have been assigned a major role in the induction of angiogenesis. By performing immunohistochemical studies of cauterized mouse corneas we investigated the *in situ* distribution of bFGF and TNF-α in the course of inflammatory neovascularization. Basic FGF was found throughout the corneal epithelium of untreated and cauterized eyes. However, it was only rarely detected in the endothelium or in macrophages prior to the onset of neovascularization. TNF-α was not expressed by any cell before the ingrowth of new blood vessels. We conclude that the release of these cytokines by infiltrating cells is of minor or no importance for the induction of sterile inflammatory angiogenesis.

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H.18 Hydroxyl radicals, generated at specific sites of human C5 by iron bound to the C5 protein, cause its activation without cleavage

W. Vogt

It was found earlier that hydroxyl radicals (OH•) generated in a fluid phase system, e.g. by iron-catalyzed decomposition of H_2O_2, convert human C5 to a C5b-like species, capable of binding C6, and of causing «reactive lysis» together with the components C7, C8, and C9 (Vogt, W. et al. 1989. Mol. Immunol. 26: 209). This phenomenon was somewhat surprising since the non-enzymic conversion of C5 probably rests upon rather specific conformational changes while oxygen radicals in general act randomly and cause non-specific damage to proteins. Evidence is now presented that iron binds to C5 (13–15 atoms of iron per molecule C5) and that oxygen radicals generated at these sites, cause the conversion, not necessarily

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accompanied by inactivation of native C5. Iron-Sepharose was prepared by treating Chelating Sepharose with $FeSO_4$, followed by washing. When C5 was incubated with the iron-Sepharose it was bound – virtually no C5 remained in the fluid phase (provided the capacity of the iron-Sepharose was not overcharged). Upon autoxidation of the iron $OH^{\bullet}$ are generated and convert the C5 to the C5b-like species. Since in the fluid phase neither C5 nor $OH^{\bullet}$. were demonstrable and since $OH^{\bullet}$ have an extremely short half-life the reaction on C5 is apparently restricted to the immediate neighborhood of the site of iron binding where the radicals are generated. In this way the reaction is directed to specific sites on the C5 molecule. In accordance with this conclusion is the fact that the $OH^{\bullet}$ scavenger, dimethyl-sulfoxide (DMSO), does not significantly inhibit the converting effect of the radicals.

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Workshop I
Immunity to Microbes, Viruses and Parasites

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I.1 Phenotypic analysis of T cells activated by various bacteria

ANKE BENDER and DIETER KABELITZ

We have previously reported (1) that inactivated mycobacteria preferentially stimulate T-cell receptor (TCR) γδ-positive T-cells among human peripheral blood lymphocytes. These results prompted us to investigate whether bacteria other than mycobacteria similarly stimulate $\gamma\delta^+$ T cells. Various gram-positive *(S. aureus, S. faecalis, S. pyogenes)* and gram-negative *(E. coli, K. pneumoniae, S. flexneri, P. aeroginosa, P. mirabilis)* bacteria as well as *C. albicans* efficiently stimulated the proliferation of E rosette-separated T cells. In contrast to *M. tuberculosis*, however, all of the above microorganisms induced growth of $TCR\alpha\beta^+$ T cells as shown by staining with monoclonal antibodies (mAb) BMA031 after 6 to 8 days of culture. When stimulated by *M. tuberculosis*, 50 to 80% of proliferating T cells were $TCR\gamma\delta^+$ as revealed by mAb TCRδ1. When IL-2 was added at the beginning of culture, a significant fraction of cells proliferating in response to bacteria were $CD2^+CD3^-$ and thus expressed an NK cell-associated phenotype. These results demonstrate that selective activation of $\gamma\delta^+$ T cells is not a general feature of bacteria but appears to be restricted to mycobacteria. Other intracellular bacteria such as *L. monocytogenes* are currently being analysed in this respect.

1. KABELITZ, D., A. BENDER, S. SCHONDELMAIER, B. SCHOEL, and S. H. E. KAUFMANN. 1990. A large fraction of human peripheral blood TCRγδ-positive T cells is activated by M. tuberculosis but not by its 65 kD heat shock protein. J. Exp. Med. 171: 667.

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I.2 Basophil histamine release in burn patients

U. BERGMANN, W. GROSS-WEEGE, F. E. MÜLLER, and W. KÖNIG

The histamine release of basophil granulocytes from severely burned patients (n = 12) after stimulation with anti-IgE or the Ca-ionophore A23187 was studied. The anti-IgE induced basophil histamine release of all patients was reduced in comparison to healthy donors beginning at day one post burn (p.b.) (5.0 ± 2.3 % vs. 30.5 ± 3.4 %), while the Ca-ionophore-

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induced release was not decreased before day two p.b. Basophils of patients who finally succumbed to their injuries showed poor responsiveness (to zero levels) over the total time. In contrast, the basophil releasability of surviving patients returned to nearly normal levels (fifth to seventh week p.b.). Already in the second week p.b. there was a significant difference in histamine release between survivors and nonsurvivors [e.g. days 6–9 p.b.: 23.7 ± 4.0 vs. 6.9 ± 2.7 ($p < 0.005$) after Ca-ionophore stimulation]. The altered basophil histamine release was neither due to a diminished dose- or a delayed time-response to the stimuli nor to differences in the basophil counts or the cellular histamine content. Our data indicate that the decrease of the basophil releasability, which may be secondary to altered signal transduction pathways in severely burned patients, correlates with the clinical outcome.

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I.3 Activation of human platelets by *Pseudomonas aeruginosa*

U. Bergmann and W. König

Clinical isolates of *Pseudomonas aeruginosa* (from burn wounds or sputa of CF-patients) were studied according to their potency to activate signal transduction (Ca^{++}-influx, protein kinase C and G-protein activation) and to release inflammatory mediators from human platelets. Bacterial culture supernatants unlike washed bacteria induce the release of inflammatory mediators [serotonin, 12-hydroxyeicosatetraenoic acid (12-HETE] from platelets. Supernatants of bacterial strains from CF patients released significantly more 12-HETE than strains from burn patients. Culture supernatants which contained high amounts of glycolipid (heatstable hemolysin) as well as the purified glycolipid induced both serotonin release and 12-HETE formation. Stimulation of platelets with glycolipid was accompanied by calcium influx, translocation of protein kinase C (PKC), activation of G-protein binding and increased GTPase activity. Bacterial culture supernatants induced PKC activation to a varying degree. A correlation to the release of inflammatory mediators was not obtained. However, the increased binding of guanylimido-diphosphate, a nonhydrolysable GTP analogue, and the activation of GTPase correlated with the amount of 12-HETE formation. Thus, our data suggest an involvement of the G-protein system in *P. aeruginosa*-induced 12-HETE release from human platelets.

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I.4 Detection of cytomegalovirus immediate early antigen by the alkaline phosphatase-anti-alkaline phosphatase technique in peripheral blood leukocytes

Andreas Bitsch, Gregor Bein, and Holger Kirchner

Recently there were several reports about the diagnosis of active cytomegalovirus (CMV) infection via the detection of CMV immediate early antigen (IEA) in peripheral blood

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leukocytes of renal transplant patients. IEA was detected very early during the course of CMV disease even before seroconversion or positive virus culture. Performance of this test took only 6 hours and sensitivity and specificity were about 90%. However, sometimes there were only very low numbers of IEA-positive cells (i.e. 1/100 000). With the indirect immunoperoxidase staining described originally detection of such cells was sometimes not reliable because of endogenous peroxidase activity. In order to avoid degradation of the antigen by inhibition of the endogenous peroxidase we decided to use the alkaline phosphatase-anti-alkaline phosphatase (APAAP) technique. In brief, leukocytes were separated by dextran sedimentation and centrifuged onto glass slides. In order to preserve an intact morphology without the loss of antigenic reactivity we tested several fixatives. Fixation with acetone/methanol (1:1) for 90 sec. turned out to be optimal. After performance of the APAAP procedure the IEA positive cells could be recognized very easily by a strong red nuclear staining without any background. Sensitivity and specificity were higher than with the technique described originally. Currently the IEA-test is used in a prospective study in renal transplantation.

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I.5 Characterization of cloned T lymphocytes specific for *Listeria monocytogenes* with functional heterogeneity in vivo

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Infections with facultative intracellular bacteria result in granuloma formation and the establishment of T-cell dependent immunity. In this study, T-cell lines and clones specific for *Listeria monocytogenes* were isolated from mice immunized with heat-killed bacteria. Our analysis of the T-cell lines and clones concentrated on the following *in vivo* and *in vitro* functions: (i) specific proliferation in response to various antigen preparations, (ii) adoptive transfer of granuloma formation in spleen and liver of naive recipient mice, (iii) adoptive transfer of systemic anti-listerial protection, (iv) local DTH induction, and (v) lymphokine production upon stimulation with *Listeria*-primed antigen presenting cells. It could be demonstrated, that *Listeria*-specific high responder T-cell clones were heterogeneous in their capacity to adoptively transfer either protection or granuloma formation. Clones mediating only protection or granuloma formation could be distinguished from those mediating both functions. No correlation between the different *in vivo* activities of these clones and their proliferative responses to *Listeria monocytogenes* could be observed. High levels of TNF-α/lymphotoxin were detected in some culture supernatants derived from T-cell clones activated with heat-killed *Listeria*-primed macrophages. Further studies will concentrate on the question, whether the *in vivo* functional heterogeneity of T-cell clones specific for *Listeria monocytogenes* can be correlated with specific patterns of lymphokine production in response to activation of the clones.

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I.6 A rabies-specific human monoclonal antibody with neutralizing activity

K. Enssle, R. Kurrle, R. Köhler, H. Müller, J. Hilfenhaus, E. J. Kanzy, and F. R. Seiler

According to a recommendation of WHO for prevention of a possible rabies infection, active vaccination has to be combined with application of immunoglobulin to get a fast protective effect before virus particles have reached nervous tissue. At present, preparations of purified human rabies immunoglobulin (HRIG) or equine rabies immunoglobulin are used. We have generated a human rabies-specific monoclonal antibody by EBV-transformation of human B cells followed by fusion with a mouse myeloma. The resulting clone TW-1 secrets a IgG1 monoclonal antibody which in ELISA reacts with rabies, but not with appropriate controls or nonrelated viruses. The antibody is reactive with the strains CVS-27, Fluri LEP, and Pitman Moore in an identical way. Western Blot analysis revealed fine specificity for gp67 of rabies. The antibody is neutralizing rabies virus *in vitro* (RFFIT) as well as *in vivo*, even in a concentration of approx. 0.1 µg/ml. In addition, TW-1 protects infected mice. Therefore, TW-1 might be very useful for passive vaccination. Compared to polyclonal human rabies immunoglobulins the standardized specificity and the very low protein amounts of TW-1, necessary for therapeutic effects, might be major advantages for clinical application.

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I.7 Suppression of proinflammatory mediator release by mucoid isolates of *Pseudomonas aeruginosa* in cystic fibrosis

P. Friedel and W. König

Mucoid as well as non-mucoid strains (13 clinical isolates) of *P. aeruginosa* from patients with cystic fibrosis (CF) were characterized according to their outer membrane protein profile as well as LPS pattern. Bacterial carbohydrate content was dependent on culture growth and time. Two hemolysins (heat labile, heat stable-glycolipid) were identified. Incubation of human granulocytes and basophils/mast cells with the bacteria and subsequent stimulation via proteinkinase C or ligand induced activation showed a suppressed release of leukotriene (LTB_4), hydroxyeicosatetraenoic acids as well as histamine. Isogenic variants of a mucoid and non-mucoid strain clearly indicated that the deactivating properties of CF-*P. aeruginosa* are due to the formation of the mucoid exopolysaccharide. Our results clearly indicate that the bacteria induced suppression of proinflammatory mediators resulting in paralyzed functions of the granulocytes may favor bacterial colonization and recurrent infection with *P. aeruginosa* in CF-patients.

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I.8 Orally administered bacterial vaccine primes local immunity in experimental animals and humans

M. Frühwirth, H. Wolf, and G. Wick

Mucosal membranes, as sites of entrance for bacterial, viral, and parasitic organisms, are places of great immunological interest in the control of infectious diseases. Bacterial and other preparations, designed for peroral administration, so called «immunomodulators», are increasingly used to improve local immunity. We have chosen to investigate such a bacterial vaccine *(Paspat-oral)*, consisting of seven different strains *(Streptococcus pneumoniae, Streptococcus viridans, Streptococcus pyogenes, Staphylococcus aureus, Haemophilus influenzae, Branhamella catarrhalis, Klebsiella pneumoniae)* which are able to prime unspecific defense mechanisms in rabbits[1], due to the induction of a specific immunity in experimental animals and man. Four groups (n=6–10) of male and female Swiss-outbred mice, aged 4–6 weeks, were immunized by gavage with different doses of *Paspat-oral* on five consecutive days. To investigate the possible induction of specific immunity, serum Ig (IgA, IgM, IgG) was estimated by a two-site immunometric assay on day 14 and 35. In the group immunized with the highest dose of *Paspat-oral* we found a significant increase of serum-IgA; IgG and IgM were not affected. In addition there was no reaction found in the remaining three groups. In agreement with those findings IgA increased in lung-homogenats of treated animals. Immunoperoxidase investigations of serial-frozen sections of Peyers plaques, showed an increase of IgA positive B cells in some animals of the high-dose group. We did not find analogous alterations in other groups. These results obtained in experimental animals were scrutinized in a preliminary human study, where five male probands were immunized with *Paspat-oral* and secretory-IgA in saliva was significantly increased after immunization.

1. Helmberg, A., G. Bock, H. Wolf, G. Wick. An orally administered bacterial immunomodulator primes rabbit neutrophiles for increased oxidative burst in response to opsonized zymosan. 1989, Infection and Immunity 57: 3576–3580.

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I.9 Stimulation of peripheral T lymphocytes with mycobacterial antigens separated by two-dimensional gel electrophoresis

Heinz Gulle, Bernd Schoel, and Stefan H. E. Kaufmann

Lymphocyte receptors are endowed with an exquisite specificity which allows the immune system to deal with a plethora of different molecules and microorganisms in a highly selective way. Besides their role in the host, antibodies have provided extremely valuable tools for the identification, isolation and purification of numerous molecules. The analytical potency of antibodies is exemplified impressively by the Western blot technique that allows identification of proteins separated by one- or two-dimensional gel electrophoresis. Similar attempts to use T cells for protein detection have been elusive. Here, we describe a device which allows the direct and rapid screening with viable T cells of complex protein mixtures separated by 2D gel electrophoresis. The device allows the transfer by electroelution of 480 distinct fractions from

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a 2D-gel into soluble phase. Transferred fractions are virtually nontoxic, thus allowing direct probing with viable cells. This approach should be applicable to the rapid identification and characterization of any interesting T-cell antigen, for example from important pathogens against which a subunit vaccine is desirable. Using this procedure we show that T cells from normal healthy individuals, contacts, and tuberculosis patients recognize a multitude of *Mycobacterium tuberculosis* antigens. Although the device was developed with the aim of defining to define T-cell antigens with potential value for vaccine design we envisage that this procedure has a much broader scope.

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I.10 POMC-gene expression in spleen cells induced by endotoxins or virus

J. Hahn, Martina Wollenberg, U. Lendeckel, R. Walther, Renate Mentel, Ingrid Klöting, and Christine Schütt

β-endorphin as an endogenous opioid peptide plays a key role in stress situations as trauma, infection, sepsis, and shock. Looking for the relatedness of changes in opioid peptide levels and the breakdown of immune defense mechanisms in septicemia we used a cDNA-probe of proopiomelanocortin (POMC) gene sequences to measure the inducibility of POMC-gene expression in rat spleen cells *in vivo* and *in vitro*. The rat POMC-cDNA sequence consists of 440 bp (β-endorphin, gamma-LPH, ACTH 9–39) and was a kind gift from W. A. Karginov, Novosibirsk, USSR. Blotting techniques were performed using ^{32}P-ATP labelling.

Rat spleen cells were cultured for 6 hours with different mitogens, bacterial products, and live viruses. PHA, PWM, ConA did not induce POMC-gene expression. Protein A (20 μg/ml) and LPS (50 μg/ml) from *E. coli* 055:B5 (DIFCO) and *E. coli* 0127:B8 (Sigma) as well as parainfluenza virus (TCID50 10^{-1}, 10^{-2}, 10^{-3}) were identified as stimulators of POMC-gene expression in immunocompetent cells. We present data that the non-T cell fraction of spleen cells seems to be the β-endorphin producers. The time course of POMC-gene expression in the spleen was studied using an endotoxic shock model in male NEDH-rats receiving 10–50 mg/kg LPS of different sources.

The ability of spleen cells to produce β-endorphin under «immunological stress» situations by itself seems to be of immunoregulatory importance for ongoing immunoparalysis.

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I.11 *Nippostrongylus brasiliensis – in vitro* Ig(G,E) production in mice

M. Hishmat, Ute Stephan, Laila Abu Basha, and W. König

Most nematode parasite infestations induce significant IgE responses. We used the infection of mice with *Nippostrongylus brasiliensis (Nb)* as a host-parasite system to analyse an IgE-mediated immune response. Stimulation of spleen and mesenteric lymph node (MLN) cells

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with different parasite preparations lead to characteristic immune responses. We investigated the modulation of the IgE and IgG production as well as the lymphocyte proliferation after incubation with different Nb preparations. We incubated spleen and MLN cells of normal mice and mice infected with Nb with worm and larval homogenates and worm and larval secretions at two different doses (10, 50 µg/ml). The IgE and IgG concentrations in sera and lymphocyte culture supernatants were determined on the 5th day of culture by ELISA technique. The rate of cell proliferation was determined at the 3rd day of culture by using the ^{3}H-thymidine-incorporation test. Our results revealed that: 1. IgE and IgG levels in mouse sera increased gradually after infection. Total IgE reached a peak (+503 %) at the 4th week, whereas total IgG reached a peak (+430 %) at the 3rd week and then gradually decreased. 2. The *in vitro* IgE production was stimulated preferentially in response to parasite preparations, which were applied to spleen and MLN cells. The IgG synthesis was inhibited by the parasite preparations. 3. Worm and larval secretions had a greater effect on the stimulation of IgE (+318 %) and the inhibition of IgG (−80 %) and cell proliferation (+727 %) than homogenates. The greatest modulatory effect on the IgE/IgG-production and the proliferation was induced by parasite preparations at a concentration of 50 µg/ml, at which most of IgE and G were stimulated and inhibited, respectively, and cell proliferation was inhibited. These results may be due to selective activation of T-helper cells during the course of *Nb* infection. Our data demonstrate, that the secretions include antigens, which had a greater effect on the immune response *in vitro* than the antigens of the homogenates.

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I.12 Antigens combined with or conjugated to lipopeptides constitute potent immunogens for *in vitro* and *in vivo* immunizations and for the preparation of human and murine monoclonal antibodies

P. Hoffmann[1], M. Jiménez-Diaz[1], M. Loleit[1], G. Jung[2], R. Grimm[3], E. Schafer[3], and Wolfgang G. Bessler[1]

Lipopeptide analogues of bacterial lipoprotein constitute polyclonal B lymphocyte activators. Combined with or covalently coupled to antigens, they act as potent adjuvants. We could show that antigens (BSA-DNP, TNP-SRBC, HIV-1 gp160 ($BH10^{303-329}$), $EGFR^{516-523}$) combined with or coupled to the synthetic lipopeptide analogue N-palmitoyl-S-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-(R)-cysteinyl-seryl (P_3CS) constitute active immunogens *in vivo* in mice. They were also able to induce an *in vitro* humoral immune response in the murine and human systems, and B lymphocytes thus activated were suitable for fusion. Thus, the antigens HIV-1 gp160 ($BH10^{303-329}$), HIV-1 gp160 ($RF^{316-341}$), HIV-2 p17 ($ROD^{111-121}$), chaperonin/phytochrome, and histamine combined with or conjugated to P_3CS could be used for *in vitro* immunization followed by the preparation of murine and human monoclonal antibodies. Our novel immunization procedure offers reproducibility, high antibody titers (often after one immunization), lack of toxicity of the adjuvants, easy chemical preparation of the conjugates in mg amounts, and the applicability of the conjugates for screening for the antibodies obtained.

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I.13 Mechanism of inflammatory mediator release and cytokine expression by microbial surface components of *E. coli* and *E. coli* α-hemolysin

B. König and W. König

Microbial surface properties as well as toxins are responsible for the release of inflammatory mediators from target cells. In this respect genetically cloned hemolysin-positive (hly$^+$) *E. coli* strains induced the release of oxygen radicals, leukotrienes, and histamine from human polymorphonuclear granulocytes (PMN) as well as from lymphocytes, monocytes, basophils (LMB). Activation of the cells by hly$^+$ *E. coli* leads to calcium influx, translocation of protein kinase C as well as to activation of G-proteins. Isogenic cloned *E. coli* strains with defined surface properties (MS-Fim$^+$, MSH$^+$; S-Fim$^+$, S-MRH$^+$, P-Fim$^+$, P-MRH$^+$) also induce mediator release provided they express S- or P-fimbriae. In contrast to the granulocyte the monocyte is deactivated for the release of low molecular weight mediators. Adherent bacteria activate the protein kinase C in both cell types while studies on G-proteins (GTPase activity, GDP-GTP exchange) indicate the involvement of different G-proteins in human PMN and LMB. Deactivation of the monocyte correlates with the occurrence for cytokine expression (m-RNA transcripts for CD23 and IL-6) as well as cytokine release (IL-6, TNF-α). Most pronounced effects are observed with *E. coli* bacteria expressing S-fimbriae as well as S-hemagglutination properties. Our results suggest that microbial surface properties and toxins activate human target cells via a defined signal transduction cascade and further that the balance of low molecular weight mediators as well as cytokines may determine the progression of an acute into a chronic inflammatory response.

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I.14 Bestatin-induced enhancement of *in vivo* phagocytosis by mouse peritoneal cell

T. Peschke, R. Recknagel, and A. Veckenstedt

A novel phagocytosis assay was developed to assess the immune stimulating activity of bestatin *in vivo*. The dipeptide bestatin was chosen as a test compound because of its known activity to stimulate endocytic mechanisms and to activate macrophages. Ten minutes after intraperitoneal administration of 10^7 FITC-labeled *E. coli* to ICR mice, the peritoneal cells were harvested and the phagocytic index of peritoneal phagocytes was photometrically determined by fluorescence. The cells were stained with methylene blue solution after formaldehyde fixation. Phagocytes without any *E. coli* as well as cells with different numbers of ingested bacteria were counted. The experimental, quantitative values of cells from untreated mice were found to correspond with the theoretical frequencies calculated according to Poisson distribution. The level of significance T between Poisson samples 1 and 2 could

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therefore be tested by statistical analysis as T = (PI1–PI2)/(PI1/N2 + PI2/N1), where PI is the mean phagocytic index of N cells. Already an i.p. dose of 50 µg bestatin per mouse i.e. about 1.6 mg/kg body mass, resulted in a significant increase in the PI over that of control mice which received PBS only. This result confirms under strict *in vivo* conditions that bestatin represents a potent immune stimulus for phagocytic cells.

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I.15 Modulation of inflammatory mediator release from granulocytes and monocytes by invasive and noninvasive bacteria

M. Pocher, J. Scheffer, and W. König

We investigated invasion and uptake of different genetically modified bacterial strains [*E. coli HB 101* (inv⁻), *pRI 203* (inv⁺), *Y. enterocolitica 108-C* (pl⁻), *108-P* (pl⁺), *L. monocytogenes NCTC 7973* (inv⁺), *SLCC 5779* (inv⁻)]. As target cells nonprofessional (HEp-2- and HeLa-cells) and professional human phagocytes (granulocytes and monocytes) were used. The adherence and invasion of the different bacteria was dependent on the presence of the invasion-gene, the invasive *E. coli pRI 203* showed maximal uptake. Internalization of bacteria into granulocytes correlated with the modulation of the respiratory burst. The ω-oxidation of the chemotactically active LTB_4 was inhibited by the invasive strains to a different degree. A decreased LTB_4-receptor expression was obtained after stimulation of granulocytes with all invasive bacterial strains, in particular the *E. coli K 12 pRI 203*. Not only newly generated mediators, but also the release of preformed mediators, e.g. histamine from rat-peritoneal mast cells was inhibited by all invasive bacterial strains. The invasive *E. coli pRI 203* inhibited the Ca-ionophore-induced histamine release up to 90 %. Bacterial invasion modulates the inflammatory responses of phagocytes which may be due to altered signal transduction or changes in phagolysosomal fusion.

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I.16 Monoclonal antibodies specific for the outer surface protein A (OspA) of *Borrelia burgdorferi* prevents Lyme borreliosis in severe combined immunodeficiency (scid) mice

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We have shown that viable *B. burgdorferi* (B.b.) organisms induce a chronic infection associated with arthritis, carditis and hepatitis in severe combined immunodeficiency *(SCID)*

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mice but not in immunocompetent mice. The disease is similar to that found in patients suffering from Lyme disease. Since the success of antibiotic therapies is questionable in Lyme disease the search for an immune therapy is indicated. We could show that *B.b.* specific polyclonal immune mouse sera (IS) as well as a monoclonal antibody (mAb LA-2) to the spirochetal outer surface antigen A (OspA, 31 kD) are able to prevent (or mitigate) the development of the disease in *scid* mice when passively transferred together with the bacterial inoculation and subsequently at 4 day intervals for 3 weeks. The protected mice did neither develop arthritis, carditis or hepatitis nor spirochetosis. In contrast, mice treated with normal mouse serum or mAb's specific for the 41 kD antigenic component for the periplasmic flagellum or other *B.b.* specific proteins (20 kD, 65 kD, 70 kD) were not protected. It was found that the protective mAb LA-2 has the isotype IgG2b and that other OspA-specific mAb's of a different isotype (IgG1) transfer only partial protection. This suggests an important role of the classical way of complement-mediated lysis in the elimination of *B.b.* killing in addition to antibody dependent phagocytosis of macrophages. This finding favours the hypothesis that the presence of OspA-specific antibodies at the beginning of the infection is important for effective eradication of *B.b.* from the host and for protection against the disease and makes OspA a good candidate vaccine for Lyme borreliosis.

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I.17 Streptococcal exoproteins elicit an abnormal lymphocyte proliferation in patients with psoriasis

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Streptococcal infections are known to trigger psoriasis. As immunologic stimuli are thought to be important in pathogenesis of psoriasis we set out to investigate the reactivity to streptococcal antigens in patients with psoriasis. Group A β-hemolytic streptococci were cultured for 9 days in soya broth. Three different preparations were obtained: 1) cytoplasmic membranes, 2) cell walls, 3) soluble products from the culture supernatants. MNL were isolated from 15 patients with active psoriasis not receiving antipsoriatic treatment and 15 age- and sex-matched healthy controls. 2×10^5 MNL were incubated for 3 and 6 days in RPMI-1640, 5 % newborn calf serum. As stimuli we used the T-cell dependent mitogen PWM (25 μg/ml) and 1, 10, and 100 μg/ml protein of the 3 streptococcal preparations. Proliferation was measured by ^{3}H-TdR uptake and expressed as stimulation index. The Wilcoxon test corrected for by Holm's procedure served for statistical comparisons. Compared to controls patients with psoriasis exhibited normal spontaneous and PWM-driven lymphocyte proliferations. Streptococcal cytoplasmic membranes and cell walls failed to stimulate MNL of controls and patients. In contrast, soluble streptococcal supernatants induced a marked stimulation of MNL. Comparing the proliferative responses of day 3 and day 6 the differences reached statistical significance in patients only, using 1 ($P = 0.0009$), 10 ($P = 0.0006$), and 100 μg/ml supernatants ($P = 0.0006$), the higher response occurring on day 6. Soya broth alone failed to stimulate MNL. We conclude that patients with psoriasis exhibit an abnormally high lymphocyte proliferation on day 6 upon stimulation with soluble products from streptococcus pyrogenes. The pathobiological importance of this phenomenon remains to be determined.

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I.18 *Mycobacterium leprae* renders Schwann cells and mononuclear phagocytes susceptible and resistant against nonrestricted killing

U. Steinhoff, A. Wand-Württemberger, and S. H. E. Kaufmann

Leprosy is a chronic infectious disease caused by the intracellular pathogen *Mycobacterium leprae*, which preferentially multiplies in macrophages and in Schwann cells of the peripheral nerves. Killing of *M. leprae* infected host cells is achieved by specific T cells of the $CD4^+$ and $CD8^+$ type, and by nonspecific killer cells of the natural- (NK) and lymphokine activated killer cell type (LAK). We analyzed the interaction between mononuclear phagocytes, Schwann cells and nonrestricted killer cells in the human system. Nonrestricted killer cells expressed lytic activity not only for K562 cells but also for mononuclear phagocytes and Schwann cells primed with killed *M. leprae*. After priming with viable *M. leprae*, both target-cell types were more resistant to lysis. Resistance was also induced after stimulation with interferon-γ or exposure of target cells to elevated temperatures (42 °C). Human Schwann cells infected with viable *M. leprae* showed increased synthesis of the 65kD heat shock protein (hsp) as indicated by Western blot analysis. These data suggest that infection of host cells with viable *M. leprae* induces hsp synthesis and that increased levels of hsp may render infected host cells more resistant to nonrestricted killing.

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I.19 Parasitic infection and lymphokines

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The infection of rats and mice with the parasitic nematode *Nippostrongylus brasiliensis* (N.b.) served as a model for studying the regulation of IgE mediated immune reactions. We analysed the effect of different cytokines and the homogenate of adult worms (WH) as antigen on the ongoing IgE and IgG synthesis. Spleen and mesenteric lymph node (MLN) cells from N.b.-reinfected mice were incubated with IL-2, IL-4, IL-5, IFN-γ, and WH in Transwell culture plates. Subsequently the IgE and IgG production were measured. The IgG secretion of isolated B cells was enhanced by the soluble WH antigens (+150 %). This effect was increased in the presence of T cells. The synthesis of IgE and IgG was inhibited by α-IL-4 and α-IL-5. In the presence of the antigen the addition of IFN-γ and α-IFN-γ suppressed the IgE production of isolated B cells (−27 % to −43 % IFN-γ, −31 % to 56 % α-IFN-γ). The incubation with IFN-γ or α-IFN-γ in the absence of WH led to an inhibition (−16 % IFN-γ) or minute upregulation (+3 %) of IgE synthesis. The IgG synthesis of isolated B cells was inhibited by IFN-γ or α-IFN-γ in the presence of WH and IFN-γ alone. Whereas the stimulation with α-IFN-γ or WH led to an enhanced IgG production (+34 % to +49 % α-IFN-γ, 34 % WH). The incubation of B and T cells with IFN-γ or α-IFN-γ in the presence or absence of WH as well as WH alone suppressed the IgE synthesis. The costimulation of B and T cells with WH and IFN-γ or α-IFN-γ enhanced the IgG synthesis (+88 %). In summary, our data show that the cytokines IL-2, IL-4, and IL-5 induce an increase of the IgE and IgG production, which could be suppressed by α-IL-4 and α-IL-5. In contrast IFN-γ and α-IFN-γ both induce an inhibitory effect on the ongoing IgE synthesis.

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